

THE ROLE OF PLANT-PARASITIC NEMATODES IN STUNTING OF PINES IN SOUTHERN PLANTATIONS

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SUMMARY

Several plant-parasitic nematode species were found associated with stunted loblolly and slash pine in plantations, the most predominant of which were lance (*Hoplolaimus coronatus*), pine cystoid (*Meloidodera floridensis*), spiral (*Helicotylenchus nannus*), and American dagger (*Xiphinema americanum*). Populations of lance and spiral nematodes were generally higher on sites exhibiting poor growth of pine than on those exhibiting good growth; whereas populations of the pine cystoid nematode were slightly higher on sites exhibiting good growth of pine.

Soils of pine nurseries were found infested with lance and spiral nematodes as well as stunt (*Tylenchorhynchus claytoni*) and stubby root (*Trichodorus christiei*). Those forms indigenous to the outplanting sites, however, were more important in the stunting of pines than those transported from the nursery in or on planting stock.

The 4 predominant species found in the survey

of plantations fed and reproduced on slash and loblolly pine. Other species found less frequently, *Belonolaimus gracilis*, *Tylenchorhynchus claytoni*, and *Meloidogyne arenaria*, also fed and reproduced on these 2 pine species.

In greenhouse tests, lance nematodes were more destructive than pine cystoid, spiral, or dagger, as measured by pine height growth and root damage. Pine cystoid nematodes caused considerable root damage but did not decrease growth as markedly as lance nematodes. Spiral and American dagger nematodes had little or no effect on growth of slash and loblolly pine.

Fumigants applied in rows at nematocidal rates in field fumigation tests prior to planting caused an increase in initial survival and growth of slash and longleaf pine. Growth of both pine species was best in rows that had been fumigated with D-D (dichloropropene-dichloropropane) at the rate of 20 gal/acre.

There are many areas in southeastern North Carolina in which new plantings of both slash and loblolly pines have failed. These areas superficially appear suitable for pine growth, but survival of seedlings in many cases has been less than 50%. The seedlings that do survive in these problem areas are stunted and annual growth is less than 50% of healthy stands. Many of these sites may be replanted for a

second or third time, but are finally abandoned because of poor survival and growth of pine.

In a preliminary survey, soil and root samples collected from plantations exhibiting poor growth contained low to moderate numbers of several plant pathogenic nematodes, whereas samples collected from plantations exhibiting good growth contained few or no pathogenic forms. The purpose of these investiga-

tions was to determine the role of nematodes in the establishment of pine outplantings and their relationship to mortality and stunting of trees in plantations. The specific objectives were 1) to study occurrence and population levels of plant-pathogenic nematodes in plantations and nurseries; 2) to determine whether those nematodes indigenous to the plantations, or those carried on the planting stock, contribute to the stunting of pines; 3) to determine which species feed and reproduce on, and affect growth of slash and loblolly pine under greenhouse conditions; and 4) to evaluate the use of certain soil fumigants for the improvement of stands and growth of pines in field plantings. A preliminary report has been given (28). Results of histopathological studies establishing the host-parasite relations of certain endoparasites are being presented in a separate paper (27).

REVIEW OF LITERATURE.—Lance nematodes (*Hoplolaimus* spp.) were first reported associated with pines (Austrian pine seedlings, *P. nigra* Arnold), in Valparaiso, Fla. by Steiner (30). Steiner (31) mentioned that a species of this nematode was found in samples from loblolly pine (*P. taeda* L.). The following year (32), he reported longleaf (*P. palustris* Mill.) and slash pine (*P. caribaea* Morelet) as hosts of *H. coronatus* Cobb, 1923 (= *H. tylenchiformis* Daday, 1905). Hopper and Ewing (16) and Hopper (15) reported lance nematodes from Georgia and Florida pine nurseries.

The cystoid nematode (*Meloidodera floridensis* Chitwood *et al.*, 1956) was described from pine in Florida (6). Hopper (15) reported this species from longleaf pine, loblolly pine, Austrian pine, and sand pine [*P. clausa* (Chapm.) Vasey]. Hutchinson and Reed (17) found this species parasitic on short leaf (*P. echinata* Mill.) and pitch pine (*P. rigida* Mill.) in New Jersey.

Spiral nematodes (*Helicotylenchus* spp.) also have been found in forest tree nurseries (11, 15). *H. nannus* was recovered from the Morgan nursery in Georgia, and a *Scutellonema* sp. from the Yulee nursery in Florida (3). A *Rotylenchus* sp. was recovered from around several eastern white pines (*P. strobus* L.) in Canada (2).

The American dagger nematode (*Xiphinema americanum* Cobb, 1913) has been considered an important parasite of pines. White (38) observed this species in 16 of 23 samples from shortleaf pine in Arkansas. Hansbrough and Hollis (11) consistently found it in pine nurseries in Louisiana. This nematode also was reported associated with eastern white pine in Canada (2) and white and red pine (*P. resinosa* Ait.) in Rhode Island (33).

Crossman and Christie (8) listed longleaf pine as a host for the bud and leaf nematode [*Aphelenchoides fragariae* (Ritzema Boz, 1891) Christie, 1932].

Buhrer (4) listed longleaf and slash pine as hosts of the root-knot nematode [*Heterodera marioni* (= *Meloidogyne* spp.)]. Turica (35) mentioned the Monterey pine [*Pinus insignis* Dougl. (= *P. radiata*

D. Don.)] as a host of this nematode in Argentina.

Root lesion nematodes (*Pratylenchus* spp.) have been reported in association with pines by Henry (14), Fischer (9), Oostenbrink (21), and Nolte and Dieter (20). Oostenbrink (22) found both *P. pratensis* (de Man, 1880) Filipjev, 1936 and *P. penetrans* (Cobb, 1917) Chitwood & Oteifa, 1952, in samples from around Scots pine (*P. sylvestris* L.) in Holland. Hopper (15) listed longleaf and loblolly pine as hosts for *P. brachyurus* (Godfrey, 1929) Goodey, 1951.

Several other plant-parasitic species of nematodes associated with pines were cited in a host list compiled by Hopper (15). These include sting nematodes (*Belonolaimus* spp.); sheath nematodes (*Hemicycliophora* spp.); ring nematode (*Criconemoidea* sp.); awl nematode (*Dolichodorus* sp.); needle nematode (*Longidorus* sp.); and stunt nematodes (*Tylenchorhynchus* spp.). Four genera, *Psilenchus*, *Tylenchus*, *Ditylenchus*, and *Aphelenchus*, suspected fungus feeders, also were listed. In a host list from Canada (2), sheath and stunt nematodes were mentioned associated with white pine and jack pine, whereas pin nematodes (*Paratylenchus* spp.) were found only in samples taken from eastern white pine. A report from Russia (39) cited *Aphelenchoidea* sp. in pine nurseries.

The proof that nematodes found in association with various species of pines can parasitize these hosts has been presented for only a few nematodes. Oostenbrink (21) demonstrated that *H. uniformis* Thorne, 1949 (= *Rotylenchus robustus*) was a parasite on *Pinus* spp. in Holland. Oostenbrink (22) also showed that *P. pratensis* and *P. penetrans* parasitized Scots pine. Oostenbrink, *et al.* (24) found that *Pratylenchus penetrans* was a pathogen on *Pinus nigra* Arnold v. *austriaca* (Hoess) A. & G., *P. montana* Willk. ssp. *mughus*, *P. nigra* Arnold v. *calabrica* (Loudon) Schneider, and Scots pine.

Steiner (31) mentioned that *Hoplolaimus* spp. damage seedlings of loblolly pine, but no experimental data were presented to support this.

In pathogenicity tests with the cystoid nematode on slash pine (*P. elliottii* Engelm.), Chitwood and Esser (5) observed no apparent damage to greenhouse inoculated seedlings. Hopper (15) stated, however, that the cystoid nematode caused severe injury and mortality to sand pine at the U.S. Department of Agriculture Forest Service Experimental Nursery at Olustee, Fla.

Hansbrough and Hollis (12) made several unsuccessful attempts to demonstrate that the dagger nematode could feed and reproduce on pine seedlings. Tarjan (33) had difficulty in colonizing the dagger nematode in greenhouse experiments. He felt that the inability to colonize this nematode did not necessarily mean that it was not a parasite but rather that greenhouse conditions were unfavorable for its growth and reproduction.

In inoculation experiments with the dagger nematode on pine seedlings, White (38) observed moderate

size swellings on young roots, clusters of short stubby branches, varying degrees of necrosis, and a shriveling of small roots at points of attachment.

Criconemooides rusticum (Micoletzky, 1915) Taylor, 1936, was found associated with the deterioration of shortleaf pine roots by Jackson (18). Parasitized portions of the fine roots became necrotic and reddish-brown. The mycorrhizae were swollen and spongy and the surface was covered with finely reticulated brown scales.

Tylenchorhynchus claytoni Steiner, 1937, and an undescribed species of this genus caused considerable damage to pine seedlings in nurseries in Mississippi and Louisiana (15). Low levels did not appear to affect pine seedlings, but when populations were 1,500-2,000/pint of soil severe injury occurred.

Other accounts of nematodes in association with pine forest tree nurseries gave only indirect evidence that the nematodes involved were pathogens (9, 12, 14, 19). Parasitic species of nematodes were present in "sickness" areas of the nurseries. An improvement in growth of a subsequent crop of seedlings was obtained by fumigating.

MATERIALS AND METHODS.—Survey of selected sites.—In the fall of 1959, a survey was conducted to determine the parasitic nematode populations in the roots and rhizosphere of pines. Five sites with excellent stands and growth of pines and 5 with poor stands and poor growth were selected in Bladen and surrounding counties. Both loblolly and slash pine plantings ranging in age from 4 to 25 years were included. Ten trees were selected at random within a 5-acre area in each site. The main lateral roots for each tree sampled were carefully spaded up and traced out to the terminal mycorrhizal clusters. The clusters and soil immediately surrounding them were collected as 1 sample/tree. The soil was well mixed and 0.5 pint processed by the elutriation method (23). The roots were washed with a fine spray of water and the washings poured through two 270- and two 325-mesh screens. The residue from these sieves remained in a Baermann funnel for 96 hours. The mycorrhizal root clusters were blotted dry and 3 g were weighed out from each sample. Each root sample was placed in 500 ml of water and cut in a Waring Blender for 30 seconds. The suspension was passed through a nest of 3 sieves—one 270- and two 325-mesh screens. The residues from the sieves were combined and placed in a Baermann funnel (1). After 48 hours, samples were drawn from the funnels and counts made of the various nematode species present.

Seedlings for the reforestation of areas in and around Bladen County come mainly from 2 nurseries. One is located at Clayton, N.C., and the other at Goldsboro, N.C. Both of these nurseries were sampled in the spring of 1958. Each sample was thoroughly mixed and rifled; 0.5 pint was placed in a plastic bucket containing 1 gal of water. This was roiled, allowed to settle for 30 seconds, and the supernatant was poured through a nest of 3 sieves—

60-, 200-, and 325-mesh. The residues were placed in modified Baermann funnels.

Reproduction studies.—Three of the most common species of parasitic nematodes found in the survey of pine plantations, the spiral nematode, the lance nematode, and the pine cystoid nematode, were cultured in the greenhouse. Spiral and lance nematodes were propagated separately on cotton, *Gossypium hirsutum* L. (var. Coker 100 WR); and cystoid nematodes on slash pine.

Five-month-old slash and loblolly pine seedlings were lifted from previously fumigated seedbeds (methyl bromide at 1 lb/100 ft²) at the Goldsboro nursery, and held in cold storage at 2°C for 1 month. A sandy loam soil, made by mixing a silt loam and sand in a 1:1 ratio, was placed in 6-in. clay pots and steamed at 80°C for 2 hours. One slash pine seedling was planted in each of 25 pots and 1 loblolly seedling each in another 25 pots. These were placed in the greenhouse at about 30°C.

The nematodes used for inoculum were separated from soil and roots by a combination of sieving and modified Baermann funnels. The nematodes were counted into lots of 500 and placed in 50 ml of water by using a micropipette apparatus described by Ford (10). Dagger nematodes also were included in this test since they were frequently found in pine plantations. Attempts to culture the dagger nematode in the greenhouse failed; therefore, a population obtained from around loblolly pine trees growing in Wilmington, N.C., was used. Because of the lack of inoculum of this species, lots of only 200 nematodes were used. Six holes were made in the potted soil about 4 in. deep, the nematode suspension was equally distributed into them, and the holes were immediately filled with moist soil. Following inoculation, the pots were randomized on a bench in the greenhouse at temperatures of 24-30°C.

At the end of 1 year, the soil from each pot was processed by the elutriation technique. Cotton-wool filters (Nematoden-Watten, 190 mm in diameter Brocades-Stheeman & Pharmacia, The Netherlands) held on screens in trays were used instead of funnels. The liquid containing the nematodes was drawn off after 24 hours; tap water was added to bring the amount for each sample up to 100 ml. Two 10-ml samples were taken and the counts averaged and multiplied by a factor of 10 to obtain the estimate of nematodes/pot.

Pathogenicity studies.—Pathogenicity of the 4 most common plant-parasitic nematodes found in outplantings exhibiting poor growth—lance, pine cystoid, spiral, and dagger—was tested on loblolly pine seedlings in the greenhouse. Two-month-old loblolly seedlings were grown in steam-pasteurized soil in a flat in the greenhouse and transplanted to 6-in. clay pots containing steamed sandy loam soil. Six treatments were as follows: 1) 400/pot, 2) 1,900/pot, 3) 9,400/pot, 4) 46,900/pot, 5) supernatant, 50 ml/pot, and 6) control. Each treatment was replicated 3 times and data were taken at the end of 6 months. Due to

insufficient inoculum, dagger nematodes were added at the rate of 450/pot and compared with supernatant and a control. This test was replicated 4 times and data taken at the end of 10 months.

Micropot test.—Since plant-parasitic nematodes were found in nurseries as well as plantations, the question arose whether the nematodes in the nursery might be carried to the plantation on planting stock or whether the species indigenous to the plantations were mainly responsible for the damage. The possibility that fungi or other pathogens may be involved either alone or in combination with nematodes in the stunting of pine seedlings also was considered. In an attempt to isolate some of these factors, a field test was conducted.

Transplantable slash pine seedlings were lifted from both fumigated (methyl bromide at 1 lb/100 ft²) and nonfumigated nursery beds, the latter being infested with approximately 200 lance nematodes/pint of soil. Because the seedlings grown in nontreated beds were generally of smaller size, all seedlings were graded to no. 2's, according to Wakeley's morphological grades (37). The seedlings were bundled in wet sphagnum moss and stored at 2°C for 1 week before planting. The site for this experiment was in the Bladen Lakes State Forest, Bladen County. This area, which had had a previous planting failure with loblolly pines and had an indigenous nematode population of those nematode parasites common in sites exhibiting poor growth, was cleared of pines and shrubs. Plots, measuring 20 x 20 ft with a 2-ft border between plots, were replicated 4 times in a random-block design. The plots were tilled with a mechanical rota-spader to a depth of 5 in. and raked level. Six treatment combinations were: 1) methyl bromide nursery/methyl bromide field; 2) methyl bromide nursery/D-D field; 3) methyl bromide nursery/nontreated field; 4) nontreated nursery/methyl bromide field; 5) nontreated nursery/D-D field; and 6) nontreated nursery/nontreated field. The methyl bromide was applied under polyethylene cover at the rate of 1 lb/100 ft². The soil temperature 6 in. deep was 15°C at time of treatment and the covers were left on for 48 hours. The D-D was applied broadcast according to the method described by Taylor (34) at the rate of 20 gal/acre. All considerations for applying soil fumigants (7) were followed. Twenty-five seedlings were planted at 3 x 3-ft intervals in the plots.

All plots were sampled for nematodes just prior to fumigation. Three samples, each consisting of 6 borings to a depth of 8 in., were processed following the modified Baermann technique. The plots again were sampled for nematodes 2 weeks following fumigation. Ten months after planting, 1 seedling was selected at random from the center 9 trees in each plot and the rhizospheres and roots were assayed according to procedures outlined in the survey section. The roots were washed and rated for color, size, and mycorrhizal development before being assayed for nematodes. At this time, certain small

laterals and short roots were selected for fungus isolations. These root sections were placed in 30% hydrogen peroxide for 10 seconds, rinsed twice in sterile water, and placed on plain agar plates. Fungus colonies were transferred to potato-dextrose agar slants 5 days later and identifications were made after 10 days. Following the second growing season, the trees were measured for height growth.

Field fumigation test.—Soil fumigation seemed a feasible approach for control of nematodes in pine plantings if row applications were used. A site having a history of several planting failures was selected for a fumigation test using 2 nematocides. The experiment was designed to measure effects of soil fumigants applied in the row on initial survival and subsequent growth of slash and longleaf pine.

A typical decline site with a Norfolk sand type soil was selected in Bladen County. This field was first planted with loblolly pine in 1954. Following a complete loss, it was again planted in 1956. Because of very poor survival and stunting of surviving trees, this site was again prepared for planting in the fall of 1957 by furrowing rows 10 ft apart to a depth of 6 in.

In the spring of 1958, experimental plots were arranged in 5 blocks of 10 rows 30 ft long. A 10-ft strip was left between each block, and the blocks were laid out in a linear arrangement across the field.

The 5 treatments used for both longleaf and slash pine were: 1) DBCP (1, 2-dibromo-3-chloropropane) 50% EC at 5 gal/acre; 2) DBCP 50% EC at 10 gal/acre; 3) D-D at 10 gal/acre; 4) D-D at 20 gal/acre; and 5) control. All rates were calculated on a broadcast basis, though the fumigant was applied only in rows. Wooden markers were placed at 3-ft intervals in the center of the furrowed row. In the center of each quadrant, 6 in. around a marker, an injection was made with a MacLean Fumigun.

Prior to fumigation, three 1-pint soil samples were collected with a sampling tube from every other furrowed row in the field, and processed by the methods used in the nursery survey.

Longleaf seedlings were obtained from untreated beds in the Clayton nursery and slash pine seedlings from untreated beds at the Goldsboro nursery and planted by hand in the treated spots 2 weeks following fumigation.

At the end of the third growing season, both slash and longleaf pine were measured for top growth. Stem diameters of longleaf pine were measured at ground level since this species normally remains in the grass stage 3-5 years.

EXPERIMENTAL RESULTS.—Survey of selected sites.—Populations of lance and spiral nematodes were generally higher in sites exhibiting poor growth than in sites exhibiting good growth; whereas populations of the pine cystoid nematode were slightly higher in good sites (Table 1). Other genera and species of plant-parasitic nematodes, such as *Dolichodorus*, *Longidorus*, *Criconemoides*, *Hemicryliophora*, *Hemi-*

TABLE 1. Predominant species of plant-parasitic nematodes recovered from rhizosphere and roots of loblolly and slash pine growing in outplanting sites

Sites	Type of pine	Year planted	Soil type ^a	<i>Hoplolaimus coronatus</i>	<i>Meloidodera floridensis</i>	<i>Helicotylenchus nannus</i> & <i>H.</i> spp.	<i>Xiphinema americanum</i>
Poor							
I	lob	1955	R1	40 ^b (1) ^c	0	82 (8)	3 (5)
II	lob	1956	Ss	0	211 (7)	15 (6)	0
III	lob	1953	Ss	56 (6)	26 (3)	46 (6)	6 (4)
IV	slash	1957	N	126 (10)	20 (7)	58 (5)	2 (3)
V	slash	1955	N	14 (10)	58 (10)	10 (6)	10 (8)
		mean		47.2	63.0	42.2	4.2
Good							
VI	lob	1953	Ps	39 (4)	134 (7)	34 (3)	0
VII	lob	1940	Nsl	0	5 (5)	0	13 (8)
VIII	lob	1957	Ps	22 (9)	66 (6)	2 (2)	3 (3)
IX	slash	1953	N	15 (10)	171 (10)	6 (5)	3 (6)
X	slash	1953	Ps	32 (3)	47 (3)	45 (10)	4 (7)
		mean		21.6	84.6	17.4	4.6

^aR = Ruston sandy loam-deep phase, Ss = St. Lucie sand, N = Norfolk sand, Ps = Portsmouth sand, and Nsl = Norfolk fine sandy loam.

^bNumber of nematodes recovered from 3 g of roots plus 0.5 pint of rhizosphere soil for each sample.

^cNumber in parentheses indicates frequency nematodes recovered out of 10 samples.

criconemoides, *Pratylenchus zeae*, *Belonolaimus gracilis*, and *Tylenchus*, were either recovered from a few samples in high numbers or were frequently found in small numbers. There was no apparent association between any of these other species and growth. Neither was there a correlation between type of pine and nematode species. Except for 1 case, the better plantations were found on sites having soil types of generally higher moisture-holding capacity.

Plant-parasitic nematodes were found in both of the state nurseries which supply seedlings for planting in southeastern North Carolina. Four species were present in soil taken from nontreated beds at the Goldsboro Nursery. Stunt nematodes (*Tylenchorhynchus claytoni*) and stubby-root nematodes (*Trichodorus christiei*) were associated with both slash and loblolly seedlings. Lance nematodes (*Hoplolaimus coronatus*) were associated with slash seedlings and spiral nematodes (*Helicotylenchus nannus*) with loblolly seedlings.

Soil samples collected from the Clayton nursery contained stunt, stubby-root, and spiral nematodes. Spiral nematodes were found in association with slash and loblolly, and stubby-root nematodes with loblolly and longleaf. Stunt nematodes were associated with slash, loblolly, and longleaf seedlings. Both spiral and stubby-root species were present in low numbers and frequency of recovery was low.

Reproduction studies.—To determine whether the several plant-parasitic nematode species found in association with pine in the outplanting sites reproduced on pine, greenhouse studies were made using known levels of inoculum of the various species.

At the end of 1 year, populations of all 4 species on both slash and loblolly were well above original inoculum levels (Table 2). With the exception of the spiral nematode, all pots containing slash pine had populations exceeding 500 nematodes. For loblolly, all pots of lance and pine cystoid had more than

the original number. With the dagger nematode, 4 of 5 pots had populations exceeding 200/pot, the original inoculum level for this particular nematode. Only 2 of 5 pots with spiral nematodes exceeded the original number.

TABLE 2. Number of plant-parasitic nematodes on slash and loblolly pine seedlings grown in the greenhouse after 1 year

Nematode sp.	Avg. no. of nemas/pot	
	Slash pine	Loblolly pine
Spiral	739	804
Lance	3210	6750
American dagger ^a	3180 ^b	1035
Pine cystoid ^c	4747	6008

^aThe inoculum level was only 200 nematodes for this species.

^bThe mean is an average of only 4 replicates because 1 seedling died after 3 months.

^cOnly counts of larvae were made with this species of nematode.

TABLE 3. Influence of various population levels of lance and pine cystoid nematodes on growth of loblolly seedlings after 6 months

Initial inoculum level ^a	Lance		Pine cystoid	
	Top/root weights (g)	Nemas/pot recovered ^c	Top/root weights (g)	Nemas/pot recovered ^c
400	6.3/3.7	2073	11.6/10.8	1077
1900	4.0/2.4	2904	11.5/13.5	553
9400	3.9/1.8 ^b	2007	3.4/3.8	213
46,900	1.5/1.1 ^b	3245	5.1/5.3	30
Supernatant				
50 ml/pot	6.7/5.5	0	11.3/13.8	0
Control	10.3/7.3	0	12.1/17.3	0

^aInoculum consisted of larvae only with pine cystoid nematodes.

^bThese represent the measurements of only 1 replication. The other plants died before the end of the test.

^cOnly nematodes from soil recovered.

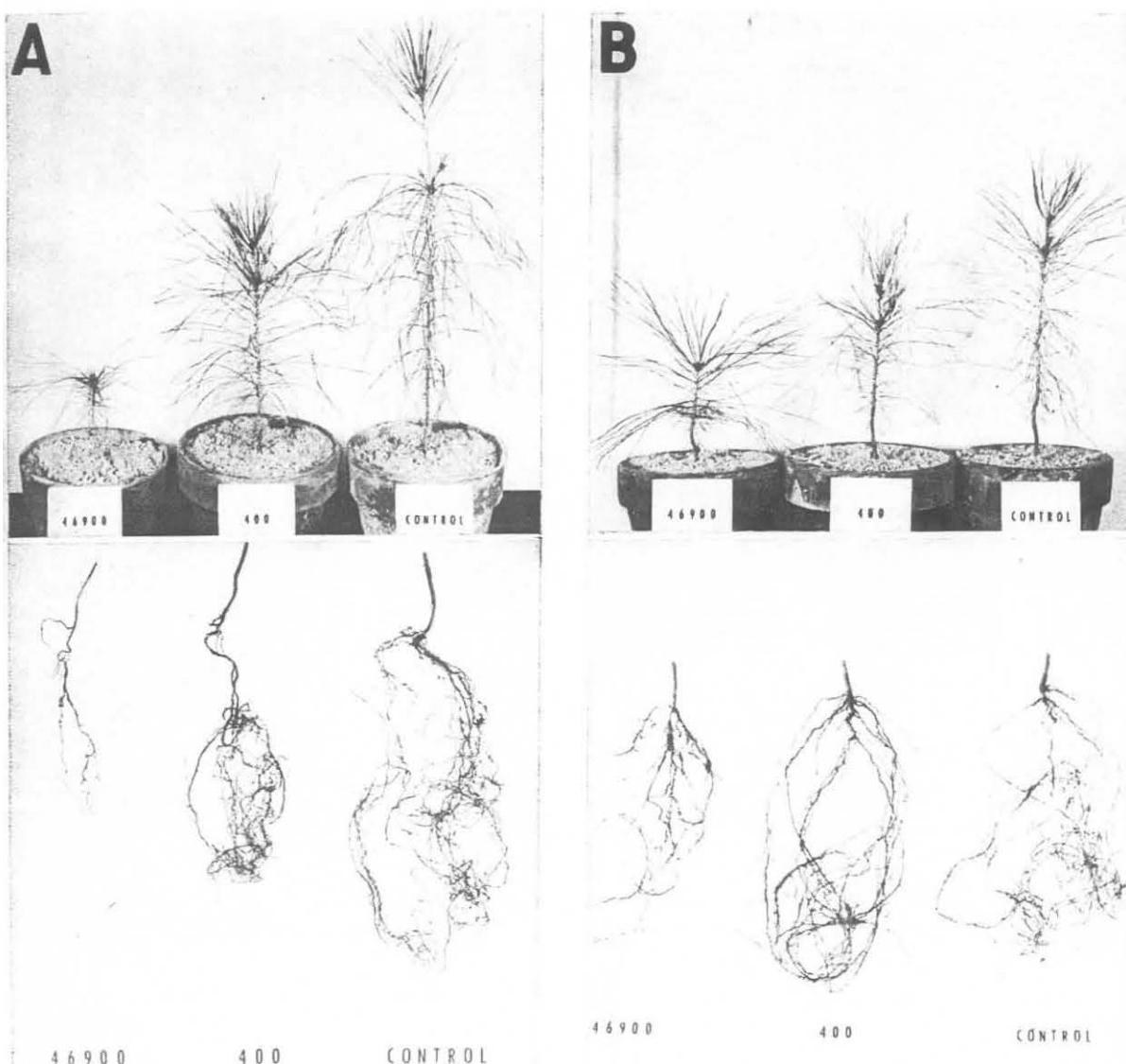


Fig. 1. Comparisons of tops and roots of loblolly seedlings grown for 6 months in soil inoculated with: A) lance nematode; B) pine cystoid nematode.

Pathogenicity studies.—The 4 predominant plant-parasitic nematodes—lance, pine cystoid, spiral, and American dagger—were studied further under greenhouse and laboratory conditions to determine the effects of the nematodes on plant growth.

The lance nematode was the most damaging pathogen of the 4 species tested. Seedlings grown in heavily infested soil were severely stunted. There was an inverse relationship between inoculum levels and seedling growth. As the inoculum level increased, top and root weights decreased (Table 3). In the 2 treatments containing the highest levels of inoculum, seedlings in 2 of 3 replicates died within 3 months of the start of the test. Root systems of the remaining seedlings, growing in the pots to which the highest inoculum levels had been added, were practically

devoid of healthy lateral roots and mycorrhizae (Fig. 1-A).

Seedlings grown in soil infested with high numbers of cystoid nematodes appeared stunted after 6 months. With increasing inoculum level, top and root weights decreased significantly (Table 3). The sparse root systems (Fig. 1-B) of seedlings grown in soil infested with the highest number of cystoid nematodes were heavily infected with mature females. These females could be seen protruding through the epidermis of both lateral and short roots (Fig. 2).

Spiral nematodes fed and reproduced on loblolly pine seedlings but caused only slight damage. The means of top and root weights were not significantly different between seedlings grown in nematode infested soil and those grown in noninfested soil. Re-

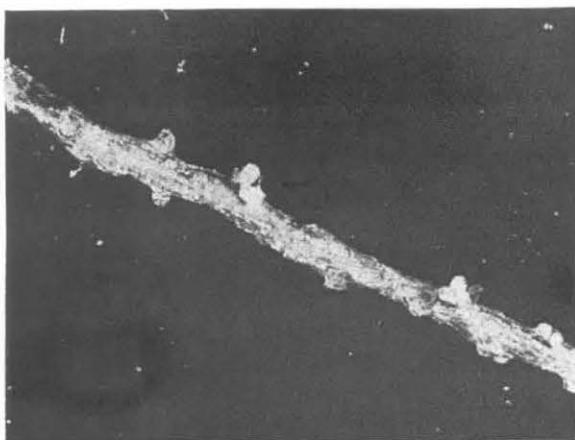


Fig. 2. Loblolly pine root infected with mature females of the pine cystoid nematode (note pearl-white body of nematode which has broken through outer layer of tissue).

gardless of initial inoculum levels, nematode populations averaged 2,500-3,500/pot at the end of 6 months. Although considerable mycorrhizal development occurred, long lateral roots did not develop on seedlings grown in soil heavily infested with this nematode.

The dagger nematode was not pathogenic on loblolly seedlings under the conditions of this test. There was no significant difference in top and root weights between any of the treatments. The nematode populations increased only slightly. Apparently, the roots were not damaged by nematode feeding.

Microplot test.—A nematode assay of the microplots prior to fumigation showed that the following species of plant-parasitic nematodes/0.5 pint of soil were rather evenly distributed over the test area: *Meloidodera floridensis*, 12 (larvae only); *Hoplolaimus coronatus*, 15; *Helicotylenchus nannus*, 10; *Xiphinema americanum*, 2; *Criconemoides* sp., trace; *Trichodorus* sp., trace. Two weeks after fumigation with methyl bromide none of the above species was present. Only a trace of the lance and pine cystoid nematodes was present where D-D was applied.

Results of an assay of soil and root samples taken from the rhizosphere 10 months after planting are given in Table 4. Where methyl bromide was used in the nursery and the planting site, no parasitic forms were found. The combination of methyl bromide in the nursery and D-D in the outplanting site reduced the population substantially below that of plots which received no treatment in the outplanting site. Where no treatment was applied, the populations of pine cystoid and lance nematodes had markedly increased in soil as well as roots. In fumigated plots, other nematode species found in the original assay at time of planting were present only in trace amounts after 10 months. In nontreated plots, they had decreased slightly.

The root systems of plants grown in soil fumigated with methyl bromide were superior to those on seedlings that were not treated (Fig. 3). The roots of plants grown in the D-D fumigated plots had well-developed mycorrhizae, especially on seedlings which came from methyl bromide fumigated nursery beds, but the fine lateral root system was not so well developed as on those from methyl bromide fumigated plots. The roots from seedlings grown in fumigated nursery beds and transplanted to nonfumigated plots were rather poorly developed and many of the lateral and short roots were dark and necrotic. Mature females of the pine cystoid nematode could be seen erupting through the epidermis of the smaller roots. Roots from seedlings which had grown in nonfumigated soil, both in the nursery and field, were in the poorest condition. The laterals were necrotic or broken off, and mycorrhizae were practically nonexistent.

The following genera of fungi were isolated from roots removed from the microplots: *Trichoderma*, *Penicillium*, *Chaetomium*, *Fusarium*, *Mortierella*, and *Gliocladium*. There was no correlation between fungus species and treatment. The first 3 fungi listed were isolated from all root samples. Of the genera found, only *Fusarium* contains species considered pathogenic.

The soil in this area was a sand of the Norfolk sand series. An analysis of the soil at the end of the

TABLE 4. Influence of soil treatment in nursery and outplanting site on nematode populations and growth of slash pine

Treatment	Nursery	Outplanting site	Nematode population ^c					
			Pine cystoid		Lance		No. of trees measured	Avg. height (cm) ^e
			Soil ^c	Roots ^d	Soil	Roots		
MB ^a	MB	MB	0	0	0	0	45	82.0
MB	D-D ^b	D-D	1	19	0	0	51	66.4
MB	None	None	146	338	13	2	41	45.7
None	MB	MB	0	0	1	0	53	88.1
None	D-D	D-D	3	7	6	0	43	58.2
None	None	None	45	815	4	0	34	32.6

^a 1 lb/100 ft² methyl bromide under cover.

^b 20 gal/acre.

^c Average number/0.5 pint of soil.

^d Average number/3 g of roots.

^e Assay made after 1 growing season.

^f Measurements taken after 2-year growth in outplanting site.

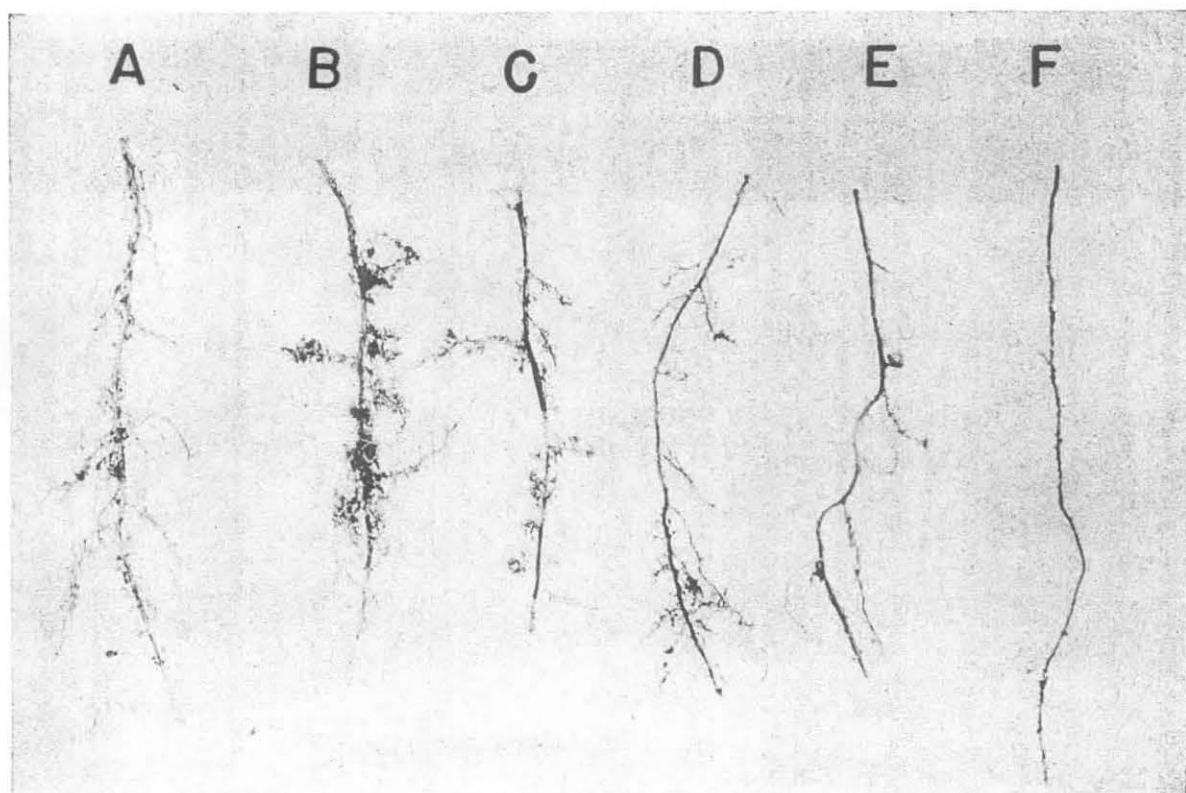


Fig. 3. Comparison of root samples taken from the seedlings removed from the microplots after 1 growing season. A) Methyl bromide nursery/methyl bromide field. B) Methyl bromide nursery/D-D field. C) Methyl bromide nursery/no treatment field. D) No treatment nursery/methyl bromide field. E) No treatment nursery/D-D field. F) No treatment nursery/no treatment field.

first growing season showed that soil fumigation had no lasting effect on pH or any of the 3 major elements.

At the end of the second growing season, height growth of those surviving trees, which had grown normally and had no tip bud injury causing bushy growth, was measured. Fig. 4, 5 show the differences in growth between the various treatments. Methyl bromide fumigation of the field plots gave the best growth (Fig. 4-A). Fumigation with D-D was not as good as methyl bromide but was still superior to no treatment. This same trend also is evident in average height measurements (Table 4). Because of the different numbers of trees measured in each plot, one LSD could not be used to test differences between means. A different LSD was calculated for each comparison. The growth of seedlings in methyl bromide fumigated plots was significantly better than those in D-D fumigated or nonfumigated plots. Those seedlings which had been lifted from fumigated nursery beds and grown in D-D treated plots were superior to those lifted from nonfumigated nursery beds and grown in D-D fumigated or nonfumigated plots. In comparing seedlings transplanted from nonfumigated nursery beds, D-D fumigation was responsible for a significant improvement in height growth.

Field fumigation test.—A fumigation experiment, designed to employ different rates of 2 nematocides in row applications, was conducted, in a field in Bladen County, N.C., having several previous planting failures, to determine if soil fumigation would benefit the initial survival and subsequent growth of pines.

Lesion, spiral, American dagger, and pin nematodes were recovered from soil samples taken from the top 6 in. of the rows after they had been furrowed out. Populations in these furrowed-out rows into which the pine seedlings were transplanted were 3-4 times less than those present in the undisturbed soil between the rows.

Of the 30 trees planted in each plot, some died and others were damaged by tip moths feeding on terminals; hence, only trees with undamaged terminal buds were measured. Because unequal numbers were used for comparison between treatments, the analysis of variance of disproportionate subclass numbers was employed (29).

Soil fumigation with D-D resulted in a highly significant increase in height growth of slash pine over those grown in nonfumigated plots (Table 5). Although an analysis of variance of the data was not made on the percentage of survival, the data demonstrate the effectiveness of fumigation with D-D on increased survival. Survival and growth were less in

those plots treated with DBCP than in the control or D-D treated plots.

Longleaf pine does not ordinarily start active height

growth until the seedlings are at least 2.5 cm in diameter at the ground line (36). Seedlings in the active growing stage will grow 30-60 cm in height

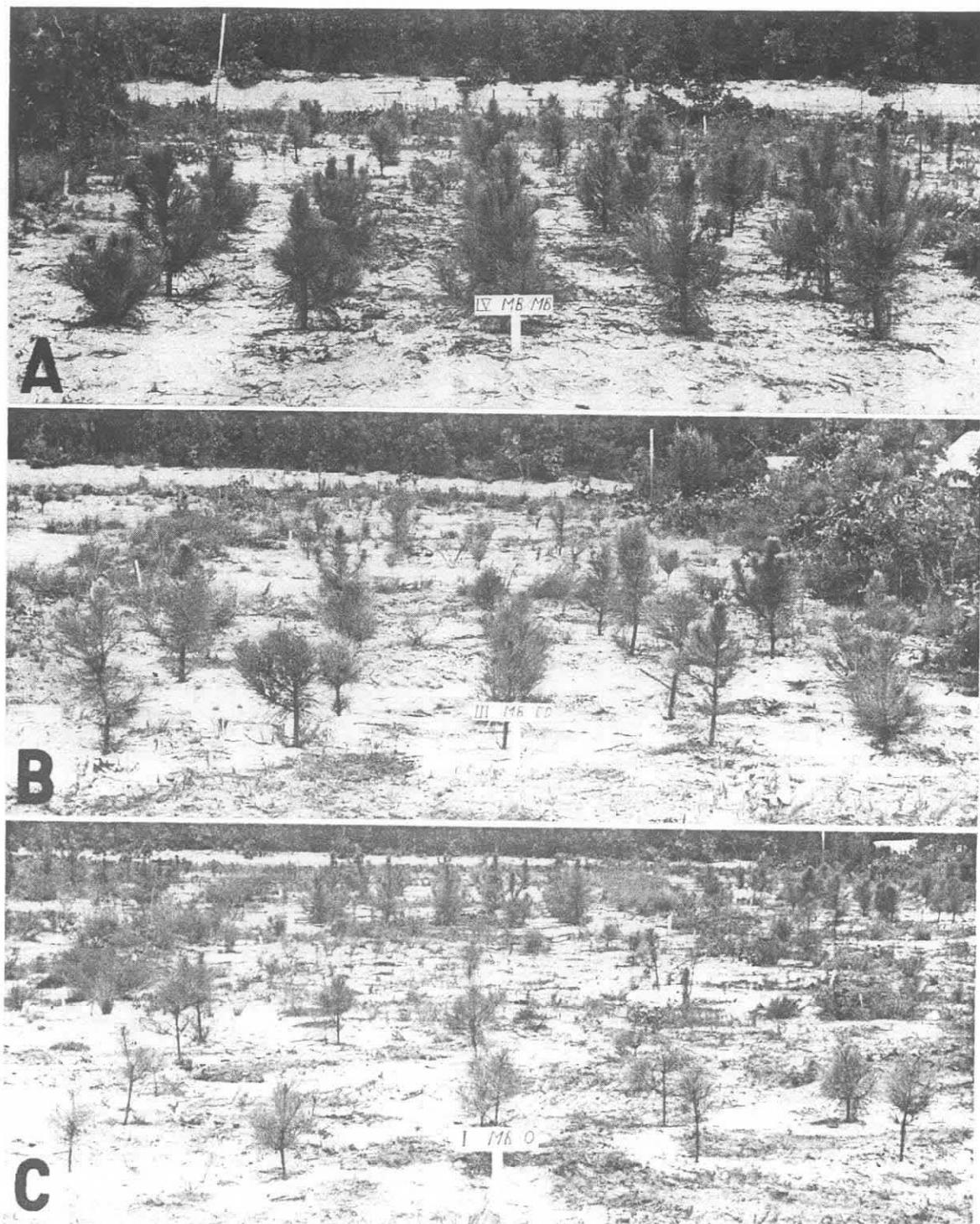


Fig. 4. Effect of soil fumigation on seedlings transplanted from methyl bromide fumigated nursery beds. A) Methyl bromide nursery/methyl bromide field. B) Methyl bromide nursery/D-D field. C) Methyl bromide nursery nontreated field.

each year. In this test, active height growth was observed only during the third year. Longleaf pine seedlings grown in plots fumigated with either rate of D-D or the high rate of DBCP significantly in-

creased basal diameter and 18-25% more of the trees started active height growth than those in the control plots (Table 5). Differences in height were not significant, but the trend was the same as in the base

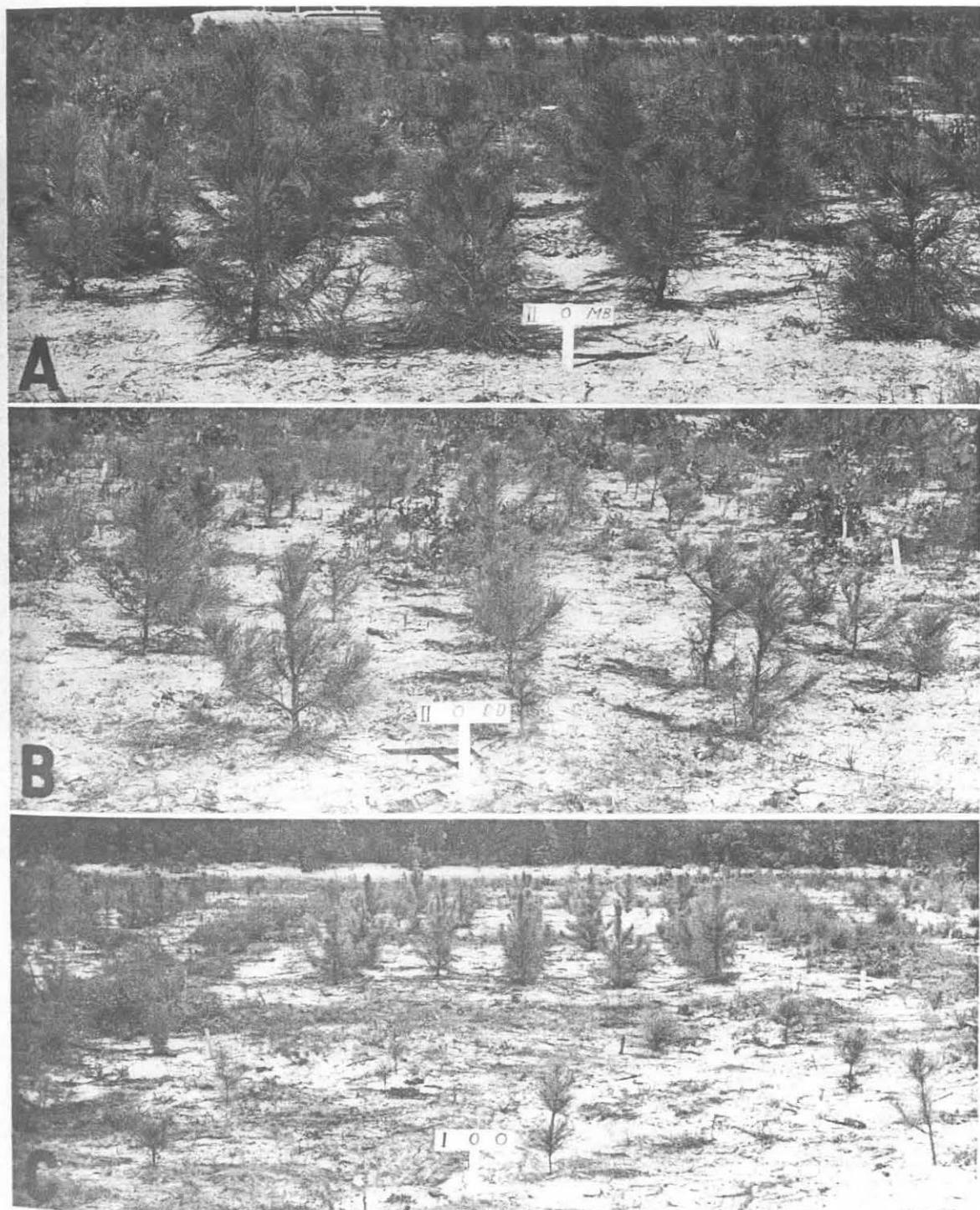


Fig. 5. Effect of soil fumigation on seedlings transplanted from nonfumigated nursery beds. A) Nontreated nursery/methyl bromide field. B) Nontreated nursery/D-D field. C) Nontreated nursery/nontreated field.

TABLE 5. Effect of soil fumigation on growth and survival of slash and longleaf pine in an outplanting site

Treatment	Slash		Longleaf			Survival (%)
	Height (cm)	Survival (%)	Mean diameter, ground level (cm)	Trees with height growth (%)	Mean height growth (cm)	
D-D at 20 gal/acre (A)	88.4 (101)*	95	3.16 (119)*	89	29.6	85
D-D at 10 gal/A	85.3 (91)	91	3.12 (120)	83	29.0	83
Control	79.2 (117)	87	2.68 (118)	64	25.3	83
DBCP at 2.5 gal/A	70.1 (79)	71	2.58 (89)	56	24.1	60
DBCP at 5 gal/A	64.0 (62)	58	2.93 (99)	82	23.8	66

* Number in parentheses indicates total number of trees measured.

diameter comparisons. There was no difference between the percent of seedlings surviving in the D-D fumigated plots and in control plots. Survival was considerably less in the DBCP fumigated plots.

DISCUSSION AND CONCLUSIONS.—Results of this study show that several plant-pathogenic nematodes occur in certain pine nurseries and outplanting sites in North Carolina. With the exception of the pine cystoid nematode (*Meloidodera floridensis*), a species which occurs only on pines, species found are common to agricultural soils of North Carolina. Most of them are serious pathogens of certain crops. The pine cystoid nematode has been reported from several southern states, but its distribution and importance are not well known.

Population levels and frequency of finding certain genera and species of nematodes in a survey of selected sites show that some are more prevalent and perhaps more important than others. For the most part, population levels for all genera were low compared with populations of these same genera commonly isolated from rhizospheres of cultivated crops. With the exception of the pine cystoid nematode, this agrees with findings of Hopper and Ewing (16). When the pine cystoid nematode was found in nurseries (15), it was usually in high numbers. The sparse root systems of pines in outplantings may account for lower population levels in contrast to a much more abundant root density in nursery beds where seedlings are grown close together (30-60/ft²) and larval counts of the pine cystoid nematode above 500/pint of soil are not uncommon. Therefore, even though individual species were few in number in many cases in outplantings, the aggregate in any 1 locality usually constituted a high population. When individual roots were dug up and samples taken from rhizospheres of mycorrhizal clusters, populations were higher than when random samples were taken around the plants.

Greenhouse tests showed that the 4 predominant nematode species found in outplanting sites fed and reproduced on both slash and loblolly pine. Increases in populations at the end of 1 year over initial inoculum levels showed that these pines were hosts.

The lance nematode was the most damaging of the species studied on loblolly pine. In greenhouse experiments, many of the seedlings died shortly after they were planted in soil containing high populations of this nematode. In those that survived, decreased

growth was directly correlated with increased inoculum levels. Roots infected with large numbers of lance nematodes turned black and eventually most of the cortex was destroyed and sloughed off.

Chitwood and Esser (5) reported that the pine cystoid nematode reproduced and increased on slash pine in greenhouse tests without apparent injury to roots or tops. They inoculated with only 2 gravid females/seedling and examined the plants after 6 months. They indicated, however, that reduction in root and top growth might be expected with higher numbers. During the present tests when high populations of the pine cystoid nematodes were allowed to parasitize loblolly seedlings for 6 months, a significant reduction in root and top growth occurred, along with considerable root necrosis.

Population levels of the various species used in greenhouse tests were well above those encountered in outplantings and nurseries. Greenhouse conditions, however, are quite favorable for plant growth and high nematode populations were necessary to show marked differences in growth. In outplanting sites, however, seedlings are often under considerable stress due to low levels of moisture and fertility. Under these conditions, relatively low numbers of pathogenic nematodes could cause considerable damage and even death of seedlings.

Much more striking differences were obtained in greenhouse pathogenicity studies when 2- to 3-month-old seedlings were used than when seedlings of transplantable age lifted from the nursery were used.

Hatch (13) stated that short roots make up 95% of all root tips in pine. Short roots of pine usually become mycorrhizal and are generally considered temporary structures. Although there still exists a controversy about the function and benefits of the symbiotic mycorrhizal relationship, the present hypothesis is that mycorrhizal roots of pine, particularly in nutrient deficient soils, are superior to nonmycorrhizal roots in nutrient absorption. If this hypothesis is correct, it then follows that any factor destroying the mycorrhizae would seriously impair normal growth of pines. Preston (25) and Robertson (26) believed that mycorrhizae also give rise to some lateral root development. They stated that many mycorrhizal-short-roots (Robertson specifically mentioned roots of *P. sylvestris*), in the spring of their second year, burst through the fungal sheath and

elongate into lateral roots. If this condition should also be common to slash and loblolly pine, then it also follows that any damage to mycorrhizae caused by pathogenic nematodes might account for some of the retarded development of lateral roots often associated with stunted pines.

Microplot tests demonstrated that those species of plant-parasitic nematodes indigenous to outplanting sites were the primary cause of poor seedling growth and not those forms transported from nurseries on planting stock. This conclusion is based on the fact that fumigation of the outplanting site resulted in increased survival and growth, regardless of nursery treatment. Failure to treat the outplanting site, however, resulted in poor survival and growth regardless of nursery treatment.

Results of field fumigation tests showed that a significant increase in survival and growth of slash and longleaf pine could be obtained with nematocidal soil fumigants. Of particular interest was the percentage of longleaf seedlings that grew out of the grass stage and began height growth during the third year in the field in fumigated plots, compared with those in nonfumigated plots. Use of fumigants to ensure better stands and more rapid growth should be given further consideration and may prove economically practical because of cost of replanting those sites where survival and growth are considered a failure. It is recognized, however, that these tests were of a preliminary nature, and that further research is needed as to fumigants, dosage levels, and methods of application.

Though the entire disease syndrome of pine stunting is more complex than one caused entirely by plant pathogenic nematodes, it is believed that the nematodes shown pathogenic here are the primary inciting agents of this stunting of pines planted on sandy sites in southeastern North Carolina.

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